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SOIL CONDITIONS IN RELATION TO LITTLE LEAF OR ROSETTE OF FRUIT TREES IN CALIFORNIA

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In an earlier paper (5) the writer referred to two factors that play the most important part in the rosette of fruit trees, namely, certain parasitic fungi and unfavorable soil conditions. The objection of Chandler and Hoagland (1) that "the conclusions reached (by the writer) are founded on a misinterpretation of certain limited observations made in the course of the general investigation and are irrelevant or inconsistent with the well established facts" is not factually supported by their published note (1). It is, therefore, difficult to guess what facts they meant. One cannot find any fact in their publications or in the literature on little leaf or rosette of fruit trees that would contradict the statements (5) made by the writer. In the present paper are included chemical analyses of water extracts from soil of affected orchards that were described in his previous article (5) to furnish on objective corroboration of his conclusions. Those figures need no further interpretation; they speak for themselves.

The fact (5) of the location of certain kinds of fungi in the bark tissues of the rosette twigs throws a new light upon the problem of that disease in this respect, namely, that it reveals some definite microorganisms and not a "virus" or another unknown agent to be responsible for the characteristic symptoms of the disease. These parasites, however, are not very virulent and are, therefore, unable to infect and destroy a tree if the climate be unfavorable for their development and the nutrition of the trees be satisfactory.

The fungi suspected of playing a causal rôle in the development of rosette of fruit trees in California need for their development a humid atmosphere; dry air, for instance, in Arizona (7), inhibits the germination of the spores on the surface of the plant organs, while a cold winter makes hibernation and development of fungi difficult, even if they succeed in penetrating

[1] The chemical analyses of the soil solutions were carried out by Messrs. J. C. Martin and R. O. Overstreet (Tables 1, 2, and 3) and H. L. Hibbard (Table 4), Division of Plant Nutrition, University of California, Berkeley, Calif., to whom the writer expresses his appreciation of their kindness.

the twig tissues. These circumstances explain why the rosette of various fruit trees is limited in the United States to certain warm, highly humid regions or, to be more specific, why it is rather common in the interior valleys of California and does not occur east of the Rocky Mountains. The fact, however, that even in some central regions of California the fruit trees remain healthy all the time, or at least for several years, indicates that the process of infection and the progress of the disease depend not so much on weather factors as they do on soil conditions.

The writer, in his studies of the cause of rosette, had opportunity to investigate affected orchards on three kinds of an alluvial, sandy type of soil, *i.e.*, a deep sand (plum orchard, Kern County), a slightly loamy sand (peach orchard, Merced County), and a loamy sand (apple orchard, Sonoma County), already described (5).

An interesting case of unfavorable soil conditions was found in the slightly loamy sand of the peach orchard, which also contained some lime. That soil was examined in all its horizons as to its microflora, soil colloids, and chemical composition of soil solution. There was found at a depth of about 6 feet a hard layer of calcium carbonate (calcareous) hardpan a few centimeters thick. The horizon of accumulation (illuvium) was located at a depth of about 4 feet; it contained, besides some loam, organic substances, hydrates of sesquioxides, and deflocculated soil colloids in a much larger amount than any other horizon. Although it was still far from being cemented into a hardpan, it was already quite impervious to water and air. Thus, between that calcareous hardpan and illuvium, an anaerobic chamber was formed in which the processes of fermentation were very strongly accentuated, mostly during the rainy season when a fresh supply of organic substances, deriving from the decaying leaves, was brought down. There was found a comparatively large amount of ferrous-ferric hydrate, which extended to just above the illuvium to a depth of about 90 to 60 cm. from the soil surface. Because of the anaerobic soil conditions, the feeder roots, formed at the beginning of plant growth, became necrotic; the xylem of the roots contained considerable iron. The fibrous roots of diseased trees were scarce and more or less covered with nematode galls. These, as well as the lenticels, weakened by anaerobiosis, exposed the roots to attack by root-invading parasites. The soil temperature, at a depth of about 3 feet, was 22.5° C. on October 24, 1931.

To ascertain the possible presence of toxins, a physiological analysis of the water extracts of that soil was carried out as follows: fresh soil samples, taken separately from each soil horizon, were stirred with an equal quantity of water and allowed to stand over night. The clear supernatant extract was dispensed into a few 1-liter corked jars, each carrying 2 sunflower seedlings about 4 cm. long; a series of 4 to 6 jars was used for the soil extract

of each horizon. Comparatively, the best nutrient value was shown by the soil extracts from the surface horizon and from the lower part of the subsurface horizon (A_2), which covered the horizon of accumulation (Table 1 and Fig. 1). Since no soil toxins could be isolated, a chemical analysis of water extracts of soil samples of various soil horizons was carried out to see whether a deficiency of some nutrient elements was responsible for a poorer growth of the sunflower seedlings in the soil extracts of the subsurface horizon and of the subsoil.

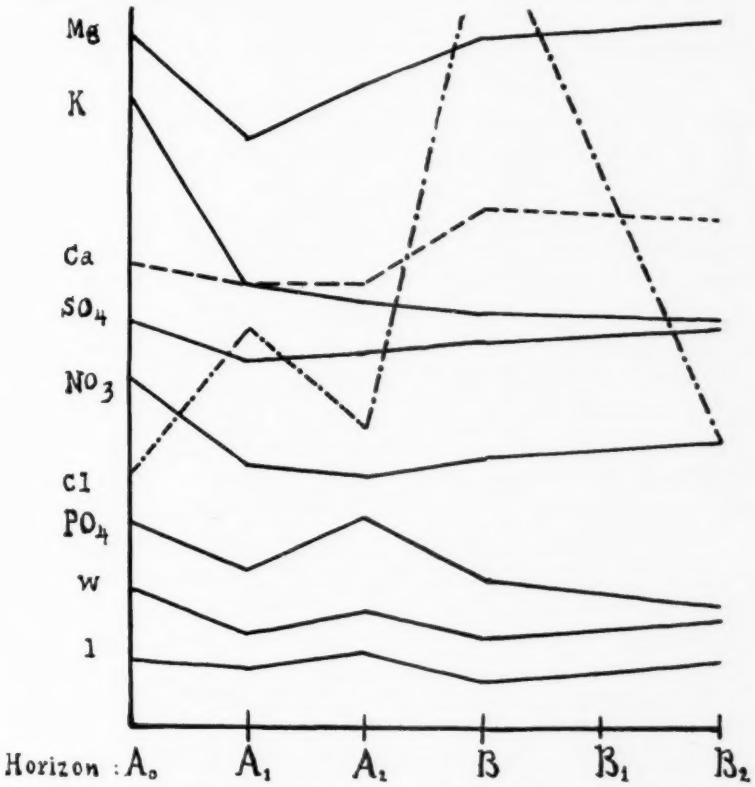

FIG. 1. Curves showing the content of certain chemical elements and of the nutrient value of the soil solution in various soil horizons. W shows average weight of dry substances, l shows average length of the stems of sunflower seedlings, grown for 2 weeks in the soil extracts.

The results of the above analysis show that a chemical composition of the soil solution is quantitatively differentiated according to the physical horizons of the soil profile. It throws also a certain light upon the processes of absorption by soil colloids; a sudden and high rise of the sodium chloride concentration in illuvium indicates that that salt was distributed in the soil

TABLE 1.—*Chemical analysis of the distilled water extracts (1:1) from the soil samples of the peach orchard in Delhi, Merced County. (The soil samples were taken in November, 1933, and the pH was determined by means of a quinhydrone electrode)*

Horizons	Depth (cm.)	pH	Components, p.p.m. in water extracts:							
			NO_3	PO_4	SO_4	Cl	HCO_3	Ca	Mg	K
A_0	1– 30	6.96	18.0	2.15	7.7	2.0	21.0	7.6	3.6	4.8
A_1	30– 60	6.96	3.6	1.6	1.4	26.0	32.0	3.9	1.8	1.69
A_2	60– 90	7.42	2.0	2.22	2.6	10.0	15.0	3.9	2.8	1.4
B	90–120	7.42	4.7	1.15	3.1	205.0	56.0	16.5	3.3	1.34
B_2	150–180	8.24	6.0	0.67	3.9	7.0	61.0	14.9	3.7	1.1

not according to the law of diffusion but rather to the law of absorption. This phenomenon is very interesting, and its explanation demands some special studies. The above analysis gave a general picture of the physiological conditions that then prevailed. A strong reduction of nitrates in the subsurface horizon indicated that anaerobic conditions were created even over the horizon of accumulation. By this means one obtained a confirmation of a microbiological soil analysis that revealed a great abundance of the denitrifying bacteria of the *Pseudomonas* group in the rhizosphere of the diseased peach trees at a depth of 2 to 3 feet.

Similar, but much more accentuated, unfavorable soil conditions were developed in a sandy loam of an apple orchard in Sonoma County. Here, too, a very compact subsoil was found at a depth of about 6 feet. The horizon of accumulation had developed at a depth of about 3 feet; it contained a considerable amount of a sticky clay besides hard lumps consisting of sand, loam, and the hydrates of sesquioxides that were cemented together. In this soil, too, there was formed an anaerobic chamber in the subsoil in which processes of fermentation were strongly accentuated, above all during the winter season. Processes of denitrification occurred even above the horizon of accumulation. Sodium chloride was accumulated in the illuvium (Table 2).

The bark of the roots of the diseased trees was in some places corroded, and that on fibrous roots was so completely decayed that only xylem remained.

A physiological analysis of the soil solution showed a very low nutrient value in all soil horizons, possibly because of a comparatively high content of the sulphates of the alkaline earths (Table 2).

A different case was represented by a deep sand in a plum orchard in Bakersfield, Kern County. It contained a small amount of loam, and was very pervious. There was found no hardpan at a depth of 6 feet, and, according to information obtained from Professor C. F. Shaw, there were

TABLE 2.—*Analysis of water extracts (1: 1) of soil samples from Sonoma County, California, taken in early February, 1934*[a]

Horizons	Depth (cm.)	pH	Components, p.p.m. (Oven-dry basis):							
			NO_3	Cl	SO_4	PO_4	Ca	Mg	K	Na
A_0	1– 30	5.55	12.8	7.0	13.8	1.4	8.4	4.0	7.3	2.7
A_1	30– 60	5.75	2.0	6.5	12.5	0.2	6.0	3.3	5.3	1.9
B	60– 90	5.15	trace	232.0	17.5	0.08	31.5	8.0	16.0	108.0
B_1	90–120	4.84	7.6	31.0	28.0	0.08	12.1	6.5	3.2	13.2
B_2	120–150	4.84	7.1	8.0	34.5	0.11	9.4	5.5	2.6	6.4
B_3	150–180	4.72	7.7	9.0	31.5	0.15	8.0	6.2	1.2	6.7

[a] pH was determined by means of quinhydrone electrode. Soil moisture was: 7.85 per cent (A_0), 8.6 per cent (B), 12.95 per cent (B_1), 14.2 per cent (B_3).

places in which no compact substratum could be found, even at a depth of 20 feet, while in some other places of that area the ground-water level reached the surface in winter. Thus, the fruit trees were exposed in that orchard to injury by a too strong fluctuation in the soil moisture content, which was very low, even during winter (Table 3). There is then no doubt that the water supply in that orchard was deficient just at the time when it was much needed. It accounts for the death of the ends of the young twigs and the lateral buds. It is possible that, in addition to drought injury, there may have been some injury chargeable to anaerobic soil conditions, because the organic products of leaf decomposition could have very readily percolated down during the winter.

In that soil the illuvium was indistinct and only slightly marked by an accumulation of small amounts of loam. Alkalinity was so pronounced that calcium carbonate incrusted the soil surface. Available nutrient matter was low in the soil solution (Table 3); and the growth of sunflower seedlings was poor in the soil extracts of all soil horizons.

Roots of the plum trees were pretty well developed, although they were often affected by canker.

The above 3 instances of a chemical analysis of the soil solution are given here to illustrate the soil conditions in the rosette-affected orchards described by the writer in his first article. If one abstains here from the discussion whether in those orchards the fruit trees were or were not subject to malnutrition because of mineral deficiency, such as that of zinc (3), in the soil solution or because the latter was not well balanced physiologically, one must, nevertheless, take into consideration some facts established by the soil analyses, namely, processes of denitrification, unfavorable soil reaction, and some unfavorable conditions in the illuvium of the orchards in which anaerobic soil conditions were strongly accentuated. The data in tables 1,

TABLE 3.—*Analysis of water extracts (1:1) of Bakersfield, Kern County soil, California*[a]

Depth (cm.)	pH	Components, p.p.m. (Oven-dry basis):								
		HCO_3	NO_3	Cl	SO_4	PO_4	Ca	Mg	K	Na
1– 30 ...	8.44	71	7.6	4	7.6	0.69	11.9	3.9	5.35	19
30– 60 ...	8.47	85	1.24	6	9.8	0.07	10.6	2.9	1.85	28
60– 90 ...	8.50	89	0.88	8	15.0	0.05	11.4	3.1	0.98	32
90–120 ...	8.58	87	0.82	6	12.3	0.07	10.6	2.7	0.83	32
120–150 ...	8.60	82	0.92	6	13.6	0.17	8.4	3.3	0.85	30
150–180 ...	8.58	84	0.86	7	14.0	0.23	8.4	3.0	0.90	33

[a] Soil samples were taken in the first days of March, 1934. Soil moisture content: 2.75 per cent (1–30 cm.), 5.9 per cent (30–60 cm.), 5.55 per cent (150–180 cm.).

2, and 3 show that in all 3 orchards soil conditions were unfavorable to normal growth of the trees. It is, therefore, not surprising that their resistance to attack by parasites was very low.

The writer attempted to verify Professor Hoagland's soil-toxin hypothesis, taking, however, into consideration also the conditions of soil and of the roots, and aboveground parts of the diseased trees. He found in his various experiments: that a rosette could not be produced either by soil microorganisms or by any product of their metabolism; that a water extract of fresh soil samples from certain soil horizons was less favorable for growth of sunflower seedlings than that from other horizons; that this less favorable condition was not traceable to an assumed soil toxin but to a deficiency of some nutrient elements, and especially of available nitrogen; that the nitrogen deficiency was due to denitrifying bacteria of the *Pseudomonas* group (type *P. fluorescens denitrificans*); and that those bacteria were much more abundant in the soil of affected orchards than in that of orchards in which the trees improved under a soil treatment; that the development of those bacteria was facilitated by anaerobic soil conditions that were distinct in the affected peach and apple orchard (Tables 1 and 2); that the roots of peach and apple trees were very badly injured, the parts aboveground showing some lesions in which were found various microorganisms; little leaves of a rosette were covered by the spores of various saprophytic or facultative parasitic fungi (*Altenaria* sp., *Cladosporium* sp., and some others), and also by numerous kinds of bacteria; and that *Monilia* sp. was found in the bark of rosette-producing twigs.

The results of all those experiments, carried out in the above order, led the writer to conclude that the trees under investigation became so weakened by unfavorable soil conditions that they could no longer resist the attack of parasites invading their roots and aboveground parts.

The hypothesis that "zinc sulphate acts by breaking up or precipitating some injurious and unknown chemical either in the soil or after absorption by the tree, or else by inhibiting the growth of microorganisms capable of producing toxic compounds" (2, p. 559) is not founded on fact, and, in the writer's opinion, is not justified because, notwithstanding various attempts, he was unable to find such toxins either in the soil or in the twigs of the diseased trees, and could not reproduce a rosette condition by any soil microorganism, directly or indirectly, or by soil extracts and fresh soil samples. That "little leaf is caused by a deficiency of zinc for normal metabolism" (3, p. 261) seemed doubtful to the authors themselves, in view of the fact that ailing trees recover often without any special treatment. The writer may add here that he has found zinc toxic to the roots of barley, even in a concentration of 0.5 mg. of zinc in one liter of nutrient solution, and there could not be found a dose of zinc that might stimulate growth of that plant.

There is no doubt that under certain conditions zinc sulphate, if applied in an adequate quantity, may improve soil conditions because it markedly precipitates the soil colloids and simultaneously changes the hydrogen-ion concentration in the soil. Thus it facilitates a proper soil ventilation, and contributes, by base-exchange and by the action of its anion (SO_4), to an increase of available nutrient elements in soil solution. At the same time, a development of anaerobic conditions and of the processes of denitrification will be inhibited. Such favorable changes in soil conditions were found in an affected peach orchard in Delhi (No. 2), described in this and in another paper, as one can conclude from the results of a chemical analysis of the soil solution summarized in table 4.

TABLE 4.—*Analysis of water extracts (1:1) of Delhi soil, Merced County, California*

Soil sample	pH	Components, p.p.m.:							
		Ca	Mg	K	SO_4	Cl	NO_3	PO_4	NH_4
A	7.4	1.7	1.0	1.0	5.0	8.0	0.6	0.2	0.1
B	5.79	7.5	1.3	4.8	15.0	8.0	2.0	2.0	0.1
C	6.76	6.0	1.7	15.3	30.0	8.0	1.6	1.6	0.0

Soil samples were taken from the same orchard (No. 2) in Delhi (Table 1) on May 10, 1933, at a depth of 2 to 3 feet, and in the vicinity of trees A, B, and C. Soil around tree A was not treated; the tree was very unhealthy, neighboring trees were dead. Soil around tree B was treated with 9 pounds of zinc sulphate in January, 1932. The tree was completely well. The soil of the neighboring trees also received zinc sulphate. Soil around tree C was

not treated. The tree bore an unhealthy aspect. Soil of some neighboring trees had been given an application of zinc sulphate.

Those figures show a distinct improvement of soil under influence of zinc sulphate in regard to the soil reaction and the content of some nutrient elements in the soil solution. (An increase of certain elements in soil solution under the influence of zinc sulphate was observed first by Krauch (6)). The analysis of soil sample C indicates that sulphuric acid, after an absorption of zinc by soil colloids, diffused quite far in the soil, and caused an increase of potassium and phosphorus content in the soil solution. Because of this circumstance, tree C started to recover (was only "partly sick"). Since Hoagland and Chandler (4, p. 271) gave no particulars concerning their method, one cannot explain why they "have not established the existence of phosphate or potassium deficiencies" in that orchard.

Deflocculated soil colloids were abundant in the nontreated soil and were completely flocculated in the treated one.

A spraying of trees with a solution of zinc sulphate (6 pounds) has, of course, the same effect as the soil treatment in some cases.

A favorable effect, were there one, of the action of zinc compounds applied through the holes into the trunk of a diseased tree might be due partly to the "fungicide" properties of zinc, which, however, are very weak, and partly to the circumstance that certain nutrient elements may be released from the xylem and made available to the buds; but, most probably, it may be explained differently.

Thus, after a critical examination of all experiments on little leaf that have been published by Chandler and Hoagland (2, 3, 4), the writer could not find a single fact in support of their hypothesis of a "specific function of zinc" or that would raise any doubt that his conclusions on the influence of faulty soil conditions upon little leaf or rosette of fruit trees in California "are irrelevant or inconsistent with well established facts." After a very careful study of little leaf for nearly 2½ years the writer came to the conclusion that that disease is due not to a supposed single factor, which has not thus far been found, but to the concerted action of various factors, such as climate, soil conditions, and microorganisms.

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BACTERIAL LEAF SPOT OF PRIMULA[1]

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A bacterial leaf spot of Primula occurs rather commonly in ornamental and commercial plantings in the San Francisco Bay region, particularly on *Primula polyantha,* Mill.

SYMPTOMS

The disease is characterized by irregularly circular brown lesions on the older leaves surrounded by conspicuous yellow halos (Fig. 1). The spots average 5 to 8 mm. in diameter and, when abundant, coalesce and kill large areas of the leaf, or even the entire leaf. In the early stages the spots are small and water-soaked, sometimes with a yellowish center, soon becoming brown and definitely delimited. When young lesions were cut across in a drop of water on a glass slide and examined under the microscope, masses of bacteria were observed to ooze out from the cut edges.

PATHOGENICITY OF THE CAUSAL ORGANISM

Cultures from young lesions yielded yellowish to greenish bacterial colonies. Healthy plants were atomized with a suspension of the organism from 48-hour-old cultures, covered with bell jars for 5 days and kept in a cool shady place. Infection developed very slowly and usually 2 weeks to a month were required for the production of conspicuous lesions. Only the older leaves proved susceptible. No infection on young leaves or on flowers was observed. Plants previously sprayed with Bordeaux 2–2–50 remained free from infection.

MORPHOLOGY OF THE CAUSAL ORGANISM

The organism is a short rod with rounded ends, and occurs singly or in pairs. It stains readily with all of the common bacteriological stains and is Gram-negative. The size of the individual cells varies considerably in any given culture, irrespective of media used, or age of the culture. In 24-hour-old cultures the cells measured from 1 μ to 3.16 μ in length and from 0.51 μ to 0.73 μ in width. The organism is motile by means of one flagellum at one pole, as demonstrated by Casares-Gil's[2] method of staining, and thus belongs to the genus Phytomonas, according to the classification of the So-

[1] Contribution from the Division of Plant Pathology, University of California, Berkeley, California.

[2] Plimmer, H. G., and S. G. Paine. A new method for the staining of bacterial flagella. Jour. Path. and Bact. **24**: 286–288. 1921.

ciety of American Bacteriologists.[3] No endospores, capsules, or involution forms have been noted.

CULTURAL CHARACTERS OF THE CAUSAL ORGANISM

Agar Poured Plates. The organism grows well on ordinary beef agar medium and more luxuriantly when 1 or 2 per cent dextrose is added to potato-peptone medium. It produces a greenish and greenish-yellow discoloration in media.[4] On peptone-beef agar of pH 6.9, colonies were visible after 24 hours' incubation at 22° C., the optimum temperature for this organism. In 36 hours, the surface colonies were round, convex, smooth, glistening, yellowish and 1 mm. in diameter. After 5 days the surface colonies reached 3 mm. in diameter; were smooth and shiny with entire margins, and there was a slight discoloration of the medium. On poured plates of potato-peptone agar containing 2 per cent dextrose, growth was much more abundant than in nutrient beef agar and discoloration of the medium was more pronounced.

Agar Stabs. The organism grew moderately along the line of puncture when deep-stabbed in beef-peptone agar, the best growth being on top in the form of a flat, circular, spreading film. Growth was better on the potato-peptone-dextrose agar.

Agar Slants. On beef-peptone agar, growth was moderate, slightly raised, smooth and yellowish in a reflected light. The medium was greenish-fluorescent in transmitted light. On potato-peptone-dextrose agar slants, growth was more abundant, and after 5 days' incubation at room temperature the color of the medium was Buffy Citrine and the growth was Andover green. After 9 days on potato-dextrose-peptone agar slants, the medium was Hazel and the slant growth dark greenish olive yellow. On potato-peptone-lactose agar slants, the medium after 5 days' incubation at room temperature was yellowish Citrine yellow; and the growth Elm green; after 9 days the medium became Citron green and the growth had a bluish hue.

Gelatin Plates. In 48 hours small, circular, yellowish colonies appeared around which later developed zones of liquefaction.

Gelatin Stabs. The liquefaction was infundibuliform in 2 days and the medium was completely liquefied in 3 weeks.

Potato Cylinders. On steamed potato plugs growth was rapid, light yellow, glistening, and spreading. The plugs had a light brown discoloration and an odor comparable to freshly plowed soil.

Milk. Milk coagulated within 3 days, and no other changes were observed after 2 weeks.

[3] Society of American Bacteriologists. Bergey's manual of determinative bacteriology. 3 Ed. 589 pp. The Williams & Wilkins Company, Baltimore. 1930.

[4] Ridgway, R. Color standards and color nomenclature. 43 pp. 53 col. pl. The Author, Washington, D. C. 1912.

Litmus Milk. Pale blue litmus milk was changed into pink and then white after 3 days when the coagulation took place. No other changes were observed after 2 weeks.

Reduction of Nitrates. No nitrites were demonstrated when the organism was grown in nitrate broth (0.1 per cent potassium nitrate in beef-peptone broth) and tested with Trommsdorf's and sulphanilic acid-α-naphthylamine reagents from 5 to 30 days after inoculation.

Ammonia Production. The organism grown in beef-peptone and beef-peptone-nitrate broths produced ammonium after 5 days, as shown by positive test with Nessler's reagent.

Indol Production. Cultures in Dunham's solution were negative for indol after 7, 14, and 21 days when tested with sulphuric acid and sodium nitrate.

Hydrogen Sulphide Production. No hydrogen sulphide was produced when the organism was stabbed and grown in the lead acetate beef-peptone agar (Difco brand).

Diastatic Action. There was no diastatic action on starch (0.3 per cent starch in beef-peptone agar) when tested after 5 to 14 days with a saturated solution of iodine in 50 per cent alcohol.

Cohn's Solution. No growth occurred in Cohn's solution.

Uschinsky's Solution. Abundant growth had occurred after 24 hours' incubation at room temperature. After 78 hours a greenish discoloration was observed at the top of the test tube, and after 1 week the medium was greenish fluorescent.

Fermi's Solution. Good growth was noted after 24 hours with the formation of a greenish fluorescence of the medium after 48 hours.

Synthetic Medium According to the Society of American Bacteriologists.[5] Good growth developed after 24 hours with greenish discoloration of the medium after 48 hours.

Toleration to sodium chloride. The organism grew very well in beef-peptone broth containing 1 per cent NaCl, fairly well in 2 per cent, weakly in 3 and 4 per cent, and was inhibited by 5 per cent of sodium chloride in the same medium.

Carbon metabolism. To test the ability of the organism to utilize organic carbon compounds, 1 per cent of dextrose, lactose, sucrose, maltose, galactose, arabinose, glycerine, dulcite, and mannitol respectively, was incorporated into S.A.B. synthetic medium[5] and incubated at room temperature. Brom cresol purple was used as an indicator. In all compounds tested, an acid but no gas was produced.

Hydrogen-ion Range. By means of adjusting beef-peptone broth with hydrochloric acid and sodium hydroxide, the following hydrogen-ion con-

[5] Society of American Bacteriologists. Com. on bacteriological technic. Manual of methods for pure culture study of bacteria. 4 Ed. Leaflet II, p. 8. 1930.

centrations were obtained: pH 4; 4.5; 5.0; 5.3; 5.5; 5.8; 6.0; 6.3; 6.5; 6.8; 7.0; 7.5; 8.0; 8.5; 9.0; 9.5; 10.0. The limit of tolerance is between pH 4.5 and 5.0, and best growth occurred at pH 6.8 and pH 7.0.

Relation to Oxygen. The organism grew in the closed arm of fermentation tubes and along the stab when inoculated into beef-peptone-agar deep. Thus the organism is a facultative anaerobe.

Temperature Relations. The organism was inoculated into broth tubes and beef-peptone-agar slants and incubated one week at 1°, 4°, 7°, 10°, 13°, 16°, 19°, 22°, 25°, 28°, 31°, 34°, 37°, and 40° C. Growth occurred between 10° and 34°, and the best growth after 24 hours was at 19° and 22° C.

Since the host plant thrives best in cool climates, it is significant that the organism grows at temperatures as low as 10° C. To determine the thermal death point, a water suspension of the organism was placed in thin-wall agglutination tubes, exposed to experimental temperatures for 10 minutes in a water bath, and cooled rapidly before inoculating fresh sterile broth with a loopful. It was found that 10 minutes' exposure to a temperature of 49.3° C. was lethal for the organism.

Effect of Freezing. To test the effect of freezing, the organism was suspended in sterile distilled water and placed in a cold-storage room at a constant temperature of 0° C. Tubes were frozen solid in ½ hour and thereafter cultured every 24 hours. The organism could not be recovered after 3 days, a fact indicating its inability to withstand exposure at 0° C.

Resistance to Desiccation. Smears made on sterile cover slips, using water suspension of a 24-hour-old agar culture of the organism, were dried for different time intervals; survival of the bacteria then was ascertained by dropping a cover slip into a sterile broth medium, with subsequent incubation at room temperature. Under the conditions obtaining, the organism survived only 60 minutes' drying. The organism survived 6 months in sterilized soil in test tubes.

Vitality in Culture Media. The organism survives 2 months in broth without any transferring and only 2 weeks in potato-peptone-dextrose agar slants.

TAXONOMY OF THE CAUSAL ORGANISM

The causal organism appears to be an undescribed species, since the morphological and physiological characters do not resemble those of any phytobacteria previously described.

Technical Description of **Phytomonas primulae** *n. sp.* A motile rod with rounded ends and one polar flagellum, single or in pairs, measuring in length from 1 μ to 3.16 μ and in width from 0.51 μ to 0.73 μ; facultative anaerobe;

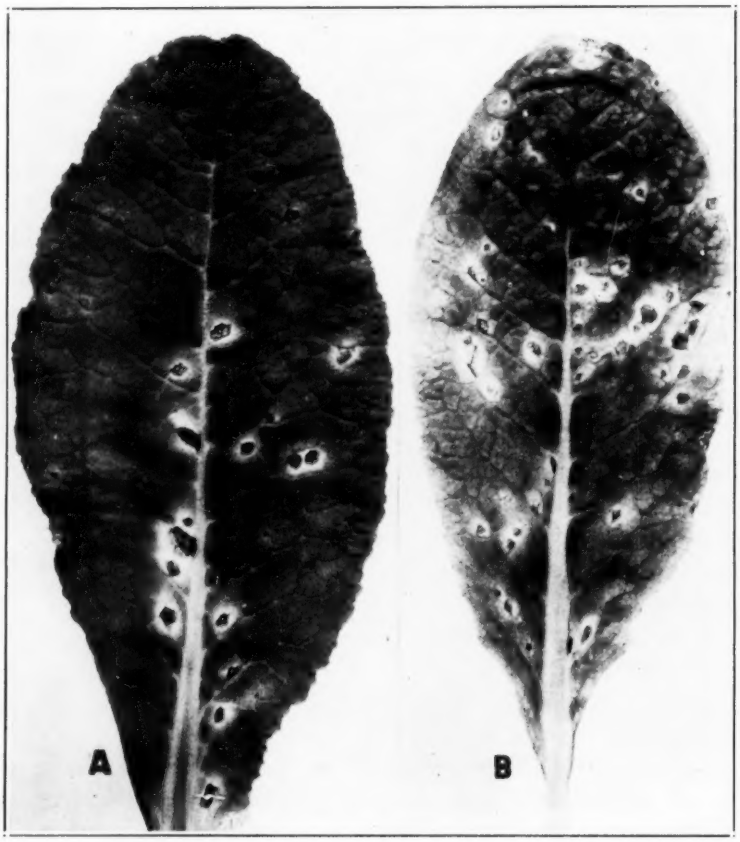

FIG. 1. Bacterial leaf spot of *Primula polyantha*. A. Natural infection. B. Infection produced by spraying the plant with a water suspension of *Phytomonas primulae*, n.sp. Six weeks after spraying.

nonspore forming; noncapsulate. Stains readily with carbol fuchsin, methylene blue, and gentian violet; Gram-negative.

On beef-peptone agar plates colonies are round, convex, smooth, glistening, margin entire; greenish discoloration of medium; produces greenish pigment in S.A.B. medium, Fermi's and Uschinsky's solutions, in beef-peptone and more pronounced in potato-lactose peptone and potato-dextrose-peptone agar; no growth in Cohn's solution. Gelatin liquefied; milk coagulated in 3 days with acid production; nitrates not reduced; indol not produced; ammonia produced; no hydrogen sulphide produced; no diastatic action on starch; acid without gas from dextrose, lactose, sucrose, maltose, galactose, arabinose, glycerine, dulcite, and manitol. Optimum temperature 19° to 22° C.; maximum 34°, minimum 10° C.; very sensitive to desiccation.

SPECIES AND VARIETIES OF PRIMULA FOUND SUSCEPTIBLE

Plants of a number of species and varieties of Primula were kindly supplied from the University Botanical Garden by T. H. Goodspeed and P. H. Brydon, for use in inoculation tests.

Infection was obtained on the following: *Primula anisodora* Balf. and Forr., *P. aurantiaca* Sm., *P. auricula* L., *P. carpathica* Fuss., *P. cortusoides* L., *P. denticulata* Sm., *P. denticulata* Sm. var. *cachemiriana* Hook., *P. elatior* Hill, *P. florindae* Ward, *P. forrestii* Balf., *P. malvacea* Franch., *P. obconica* Hance, *P. polyantha* Mill., *P. puchella* Franch., *P. secundiflora* Franch., *P. septemloba* Franch., *P. serratifolia* Franch., *P. sikkimensis* Hook., *P. violacea* Sm. and Ward, *P. vittata* Bur. and Franch., and a hybrid between *P. bulleyana* Forr., and *P. beesiana* Forr.

No infection was obtained upon *Primula japonica* Gray, *P. littoniana* Forr., *P. microdonta* Franch., *P. rosea* Royle var. *grandiflora* Hort., *P. sibirica* Jacq., and *P. viali* Del., ex Franch.

On outdoor plantings of the above mentioned susceptible species and varieties in the Botanical Garden, infection was noted on *P. aurantiaca*, *P. elatior*, *P. polyantha*, and *P. septembloba*. In a lath house, infection was noted on *P. chungensis* Balf. and Ward.

SUMMARY

The bacterial leaf spot of Primula is characterized by brown spots with yellow borders on the older leaves.

The causal organism has been named **Phytomonas primulae,** n. sp., of which a technical description is given. Colonies on agar media become green in color. The organism grows well at the low temperatures favorable to Primulas.

Among 27 species and varieties of Primula inoculated, 21 proved susceptible. Under natural conditions infection has been noted on 5 species, one of which was not included in the inoculation tests. In inoculation tests and in the garden only the older leaves proved susceptible.

INFECTION STUDIES WITH SCLEROTINIA FRUCTICOLA ON BRUSHED AND NONBRUSHED PEACHES

M. A. Smith

(Accepted for publication March 12, 1936)

The extent to which peach pubescence holds spray residue, dirt, and other foreign material is well known. In order to meet the demand for a more attractive pack, the removal of pubescence by means of brushing machines of various types has come into use in many peach packing centers.

Dorsey and Potter,[1] in their study of the structure and pubescence of the peach in relation to brushing, concluded that the peach hair is broken off at the surface of the fruit, either in brushing or handling, because the point of greatest constriction, and, consequently, the weakest point, is just below the surface (Fig. 1, B). They suggest that spores could make a much more direct contact with a brushed than with a nonbrushed surface.

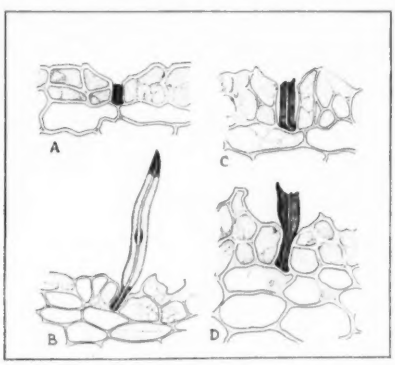

Fig. 1. A, C, and D. Types of breaks in peach hairs as a result of brushing. B. Unbroken peach hair, showing base and the buffer cells of the epidermis.

Because of the type of surface exposed (Fig. 1, A, C, D), it appears that rapid penetration and subsequent infection of the brushed fruit is possible. The work herein reported was carried out with a view to determining the manner in which the brown-rot fungus, *Sclerotinia fructicola* (Wint.) Rehm, infects brushed and nonbrushed peaches, respectively, and to ascertain differences in the ease and degree of infection.

INFECTION STUDIES

Eighty market-ripe Elberta peaches, which had not been sprayed for 30 days previous to picking, were divided into 4 lots of 20 peaches each.

[1] Dorsey, M. J. and J. S. Potter. A study of the structure of the skin and pubescence of the peach in relation to brushing. Illinois Agr. Expt. Sta. Bull. 385. 1932.

In 2 of the lots the excess pubescence was removed by brushes of a texture similar to those used in commercial brushing machines. The remaining 2 lots were not brushed. One lot of the brushed and one lot of the nonbrushed fruits were sprayed with a conidial suspension of *Sclerotinia fructicola* and then placed in damp chambers. The 2 remaining lots were not inoculated but were sprayed with distilled water and placed in damp chambers to serve as checks.

Beginning 4 hours after inoculation, and on each succeeding hour, the fruit was examined to note progress of infection. In addition, at the end of 4, 4½, and 5 hours, and for each succeeding hour thereafter, small pieces of tissue were cut from each peach and "fixed" for further study.

As brought out in table 1, in which the results of the inoculation experiments with the brown-rot fungus are summarized, infection was first observed on two of the brushed fruits after a period of 4½ hours. Five

TABLE 1.—*Infection of brushed and nonbrushed peaches by Sclerotinia fructicola*

No. fruit	Treatment	Number peaches showing infection after									
		4 hrs.	4½ hrs.	5 hrs.	6 hrs.	7 hrs.	8 hrs.	9 hrs.	10 hrs.	11 hrs.	12 hrs.
20	Brushed inoculated	0	2	5	11	16	20				
20	Brushed not inoculated	0	0	0	0	0	0	0	0	0	0
20	Not brushed inoculated	0	0	0	0	0	6	13	20		
20	Not brushed not inoculated	0	0	0	0	0	0	0	0	0	0

showed infection at the end of 5 hours, 11 at the end of 6 hours, 16 at the end of 7 hours, and 20 at the end of 8 hours. In the nonbrushed fruits infection was not observed until at the end of 8 hours, when 6 showed infection. The number had increased to 13 at the end of 9 hours, and all were infected by the end of 10 hours. It is apparent from these results that infection occurred more rapidly in the brushed than in the nonbrushed fruit.

HISTOLOGICAL STUDIES

Pieces of tissue that had been removed at hourly intervals from inoculated and noninoculated, brushed and nonbrushed fruit, were removed from the fixative and embedded in paraffin. The sections were cut 6–10 μ thick and stained with a haematoxylin-eosin stain.

Nonbrushed Fruit. In sections from nonbrushed material that had been fixed at intervals of 4½, 5, and 6 hours after inoculation, respectively, conidia could be seen held above the surface of the fruit by the hairs and, where germination occurred, the germ tubes in many cases appeared to extend out in no particular direction. Some conidia were seen resting between hairs with their germ tubes extending to or over other hairs. Occasionally one or more germ tubes were seen appressed to the sides of hairs with their tips pointed in the direction of the hair sockets.

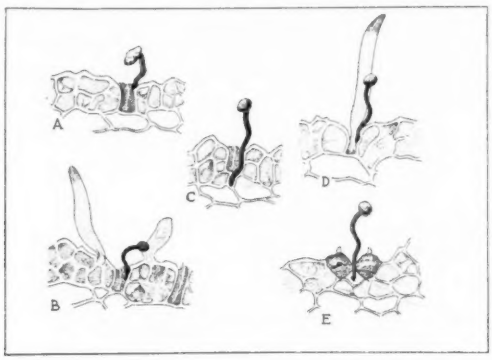

FIG. 2. A. Germinating conidium with tip resting at a point between upper edge of broken hair stub and adjacent buffer cell. B. Germinating conidium resting on short hair with germ tube pushing down between edge of hair stub and adjacent buffer cell wall. C. Penetration of germ tube through point of attachment of hair stub into tissue beneath. D. Germ tube descending hair on which conidium is resting. Note penetration of germ tube into hair socket at hair base. E. Stomatal infection. In A, C, and E spores were pulled away from epidermis during manipulaton.

In the material fixed at intervals of 7, 8, and 9 hours, respectively, the course of the germ tubes was more easily traced. By far the larger number of germ tubes could be seen appressed to the sides of the hairs, descending toward the hair sockets, then into the V-shaped depression at the base of the hair socket (Fig. 2, D).

Some germ tubes could be seen extending from one hair to another and then abruptly turning downward along the second hair to its base. Multiple infection through one hair socket was common, and germ tubes were easily traced into the tissue beneath.

Entry of germ tubes through stomata was rare (Fig. 2, E). Several instances were recorded of infection occurring through hair sockets adjacent to uninvaded stomata.

Direct infection through cuticle of epidermal or buffer cells was observed in only one instance.

From these observations it was apparent that by far the larger number of infections in the nonbrushed fruit was by way of the hair sockets, which results are in agreement with those obtained by Curtis[2].

Brushed Fruit. It would seem that conditions should be favorable for rapid infection on the surfaces of brushed fruit, since conidia that alight on the brushed surface are in very close contact with the short or broken hairs or may actually be lying in broken hair sockets or on buffer cells between hair sockets.

An examination of the material fixed 4 hours after inoculation showed that by this time large numbers of conidia had started to germinate. Measurements of 200 germinated spores at this stage showed that germ-tube length averaged 17 μ.

Sections from peaches that became infected 4½ and 5 hours, respectively, after inoculation showed germinated conidia resting on broken hair stubs with their germ tubes lying parallel to the fruit surface. Others lay on buffer cells with the tips of the germ tubes extending to the ends of broken hair stubs; and in some cases the tips had pushed in between the side of the broken hair stub and the adjacent buffer cell wall (Fig. 2, B). Examination of the material fixed 6 or more hours after inoculation showed that numerous infections had occurred. At this stage the germ tubes were, as a rule, easily traced as they grew downward along the broken hair stubs (Fig. 2, A), between the stub and the adjacent buffer cell to the point of attachment of the stub and then into the tissue beneath (Fig. 2, C).

Stomatal and direct infections, as with the nonbrushed fruit, were rarely seen. An examination of several hundred slides showed an average of 40 infections by way of broken hair sockets to 1 directly through the epidermis or through a stoma.

While hair-socket infection was also the predominant type in the nonbrushed fruit, it was apparent that infection of the brushed fruit occurred more promptly and that the rapidity with which this infection occurred was correlated with the relative accessibility of the brushed fruit surface to germinating conidia of *Sclerotinia fructicola.*

SUMMARY

Methods of infection of brushed and nonbrushed peaches by *Sclerotinia fructicola* were studied.

Infection of the nonbrushed surface of the peach variety Elberta occurs mainly by way of the hair sockets. The minimum period for infection was 8 hours.

[2] Curtis, K. M. The morphological aspect of resistance to brown rot in stone fruit. Ann. Bot. [London] 42: 39–68. 1928.

The most common method of infection of brushed fruit was by way of the broken hair sockets. The minimum time for infection was $4\frac{1}{2}$ hours. Stomatal and direct infections were not common either in the brushed or the nonbrushed fruit.

The rapidity with which infection occurs on the brushed fruit is apparently correlated with the relative susceptibility of the brushed fruit surface to germinating conidia of *S. fructicola*.

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EFFECT OF LEAF-HOPPER YELLOWING UPON THE CAROTENE CONTENT OF ALFALFA[1]

HOWARD W. JOHNSON[2]

(Accepted for publication June 2, 1936)

INTRODUCTION

Second-cutting alfalfa throughout the Eastern and Mid-western United States is yellowed and stunted to a greater or less extent each summer by attacks of the potato leaf hopper, *Empoasca fabae* (Harris). In a previous paper[3] the writer reported that yellow alfalfa is lower in nitrogen and higher in carbohydrates than green alfalfa kept free from leaf hoppers. Since the protein content of alfalfa is one of the chief measures of its quality, this represents a loss that growers suffer in addition to the obvious reduction in yield. Recent research has shown that the vitamin A activity of animal roughages is another important measure of their quality. Since the carotene content of plant tissues is now recognized as a measure of their potential vitamin A activity, an experiment was planned to secure data on the effect of leaf-hopper yellowing upon carotene content of alfalfa.

EXPERIMENTAL METHODS AND RESULTS

Samples for the writer's previous analytical work were produced in infested and noninfested cages of cheesecloth or tobacco cloth. To avoid the altered light and moisture conditions to which caged plants are subjected, it seemed wiser in this experiment to adopt some method of protecting certain field plots of alfalfa, while leaving others unprotected. The most convenient method of protection appeared to be dusting with a mixture of 300-mesh sulphur and pyrethrum, as recommended by DeLong[4] for the control of this insect on beans and potatoes.

The alfalfa plots used were sown in September, 1933, and were in their second cutting season at the time of this experiment. The plots were cut

[1] This paper reports the results of one phase of a cooperative study of the injury by *Empoasca fabae* (Harris) to forage legumes being made by the Division of Cereal and Forage Insect Investigations, Bureau of Entomology and Plant Quarantine and the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Indebtedness is acknowledged to C. A. Cary and H. G. Wiseman, Bureau of Dairy Industry, U. S. Department of Agriculture for making the carotene determinations.

[3] Johnson, H. W. Nature of injury to forage legumes by the potato leafhopper. Jour. Agr. Res. [U. S.] 49: 379–406. 1934.

[4] DeLong, D. M. The relative value of Bordeaux mixture, sulphur and pyrethrum products in reducing populations of the potato leaf hopper (*Empoasca fabae* Harris). Jour. Econ. Ent. 27: 525–533. 1934.

on May 28, 1935. Starting on June 18, when recovery growth was well under way and slight yellowing was evident, one plot of Grimm alfalfa was dusted once each week for 4 consecutive weeks, while a duplicate plot was left unprotected. By July 9, when the last dust application was made, the unprotected alfalfa was severely yellowed and stunted, while the dusted alfalfa was green and vigorous. This difference is shown in figure 1, which illustrates the dusted and nondusted alfalfa on July 15, 1935. On July 17, 300 gram samples from the two plots illustrated in figure 1 were weighed in the field and plunged immediately into a

FIG. 1. Plots of second-cutting Grimm alfalfa growing at Arlington Experiment Farm, Rosslyn, Virginia, on July 15, 1935. Both plots were cut on May 28 and, starting on June 18, the plot on the right was dusted once each week for 4 consecutive weeks with a mixture of 300-mesh sulphur and pyrethrum, while the plot on the left was left unprotected. The dusted alfalfa is green and vigorous, while the nondusted is severely yellowed and stunted by a heavy infestation of potato leaf hoppers.

freezing mixture of absolute alcohol and solid carbon dioxide. The samples thus prepared were taken to the Nutrition Laboratory, Bureau of Dairy Industry, for carotene determinations.

The plots were cut for the second time on July 19, 1935. At this time 3- to 4-pound samples were placed in cloth bags and taken to a hay drier. When thoroughly air-dried, these samples were ground in a Wiley mill and stored in Kraft bags until December 12, 1935. On this date samples of the meal made from the dusted and nondusted Grimm alfalfa were tested for carotene. The determinations were made spectrophotometrically. The results of the two sets are presented in table 1.

It is seen from the first set of determinations in table 1 that the fresh green alfalfa contains 262 mg. of carotene per kilo of dry material, while the leaf-hopper-yellowed alfalfa contains only 105 mg. The second set of determinations show that after approximately 5 months' storage in Kraft bags in a hot attic, the meal made from green alfalfa still contains 50 mg. of carotene per kilo of dry material, while the carotene content of the meal made from yellowed alfalfa is only 28 mg. per kilo. While loss of

TABLE 1.—*Effect of leaf-hopper yellowing upon the carotene content of second cutting Grimm alfalfa at Arlington Experiment Farm, Rosslyn, Virginia in 1935*

Plant material	Condition	Moisture	Carotene[c]
		per cent	*mg.*
Green alfalfa[a]	Fresh 7/17/35	72.83	262
	Dried, ground and stored 12/12/35	9.10	50
Yellowed alfalfa[b]	Fresh 7/17/35	72.33	105
	Dried, ground and stored 12/12/35	9.60	28

[a] Dusted four times with a mixture of 300-mesh sulphur and pyrethrum between first and second cuttings.

[b] Not dusted.

[c] Per kilo of dry matter.

carotene apparently has proceeded more rapidly in the green alfalfa under these storage conditions, yet this material still retains an amount slightly exceeding the average for U. S. No. 1 alfalfa hay, as reported by Meigs.[5] It would appear from these determinations that severely yellowed alfalfa contains only about one-half the carotene that is present in green alfalfa. This represents a further serious annual loss that must be attributed to the activities of the potato leaf hopper.

SUMMARY

Samples of green and leaf-hopper-yellowed Grimm alfalfa were taken from a plot dusted 4 times with a mixture of 300-mesh sulphur and pyrethrum between the first and second cuttings and from a similar plot left unprotected during this period.

The fresh green alfalfa contained 262 mg. of carotene per kilo of dry matter while the leaf-hopper-yellowed alfalfa contained only 105 mg.

After 5 months' storage in Kraft bags in a hot attic, meal made from the green alfalfa contained 50 mg. of carotene per kilo of dry matter, while the meal made from the yellowed alfalfa contained only 28 mg.

It would appear from these determinations that leaf-hopper-yellowed alfalfa contains about one-half the carotene in green alfalfa and is, therefore, only about one-half as rich in potential vitamin A activity.

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[5] Meigs, E. B. Vitamin A value of plant feeds fully accounted for by their carotene content. U. S. Dept. Agr. Yearbook 1935: 324–326.

A SIMPLE DEVICE FOR RECORDING THE TIME AND DURATION OF RAINFALL

RAY R. HIRT[1]

(Accepted for publication February 29, 1936)

During epidemiology studies it is often desirable to obtain, automatically, records of the time and duration of rainfall directly at the experimental area. When the location of such areas prohibits the use of electric power, either due to the absence of power lines or inconvenience in bringing in batteries to run electrically driven instruments, mechanically actuated devices must be resorted to. An ombroscope may serve such a purpose, but those described in the literature[2,3] are difficult to construct because of their complicated designs. While conducting studies concerned with the relation of meteorological conditions to the natural infection of white pine by *Cronartium ribicola* Fischer, a simple, inexpensive instrument was devised to record automatically the time and duration of rainfall. Because this instrument was so easy to construct and worked so efficiently, it was felt that it might prove useful in other research projects and therefore justified a brief description.

The device consisted of a revolving disk actuated by the mechanical parts of a standard alarm clock and the whole mounted in a suitable wooden case (Fig. 1). The wooden case was 9 inches square by $2\frac{1}{2}$ inches deep and was provided with a removable cover fashioned from heavy roofing paper. Legs, 2 inches long, were attached to the bottom of the box in order to raise it slightly above the ground or other substratum. Several quarter-inch holes were bored in the bottom of the box to permit rain water to drain away, and the entire box was painted with a waterproof varnish. The clock works were removed from their original metal case and mounted in the wooden box in such a manner that the winding key and set key projected out through the bottom. A strip of thin rust-resistant metal 2 inches wide was fastened about the clock works, so that it simulated the metal sides of the clock before the works were removed. A thin brass disk, 8 inches in diameter, was then substituted for the hour hand (Fig. 1).

[1] This work was done by the writer while employed as agent in the United States Department of Agriculture, Division of Forest Pathology, in cooperation with The New York State College of Forestry, Syracuse, N. Y.

[2] Fergusson, S. P. The ombroscope, an instrument for determining the time and duration of rain. Quart. Jour. Royal Meteorological Soc. **31**: 313–316. 1905.

[3] Covert, R. N. Meteorological instruments and apparatus employed by the United States Weather Bureau. Jour. Optical Soc. Amer. and Rev. of Scientific Instru. **10**: 345–346. 1925.

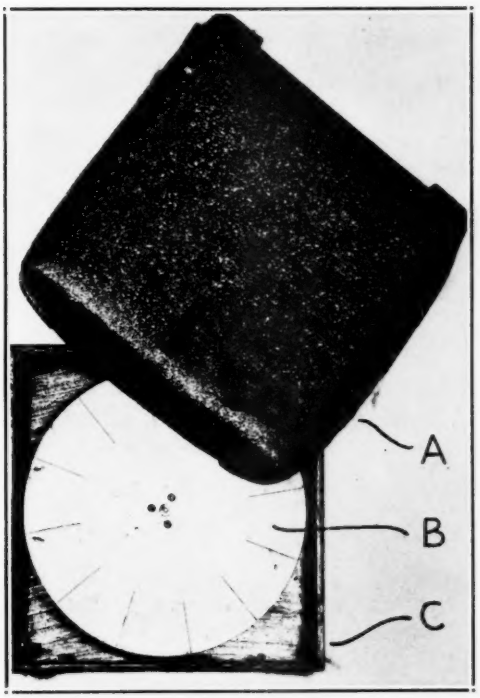

FIG. 1. The rain-recording instrument with its cover removed. A. The cover showing the slit through which rain drops may fall. B. The revolving brass disk upon which the record sheets are attached. C. The wooden box within which the mechanical parts are mounted.

Record sheets (Fig. 2), which lacked the outer concentric circles, were mimeographed on hard bond paper. Three concentric circles were then drawn in with indelible lead near the outer edge of each record sheet. Each sheet was divided into 12 equal sectors by means of heavy radial lines. Thus, when placed on the revolving brass disk, it took each sector 1 hour to pass beneath any given stationary point. The sectors were bisected with lighter lines in order to represent half-hour periods. The lines were numbered counter clockwise. The record sheets, which were of the same diameter as the brass disk, were held in position on the disk by means of 2 paper clips.

A slit 2 inches long was cut in the cover of the box directly over the revolving disk such that the outer edge of the slit corresponded with the edge of the disk. Each end of the slit was made equal in length to 1/48 of the circumference of that part of the disk directly beneath it. Hence it took an interval of 15 minutes for 1 radial line of the record sheet to

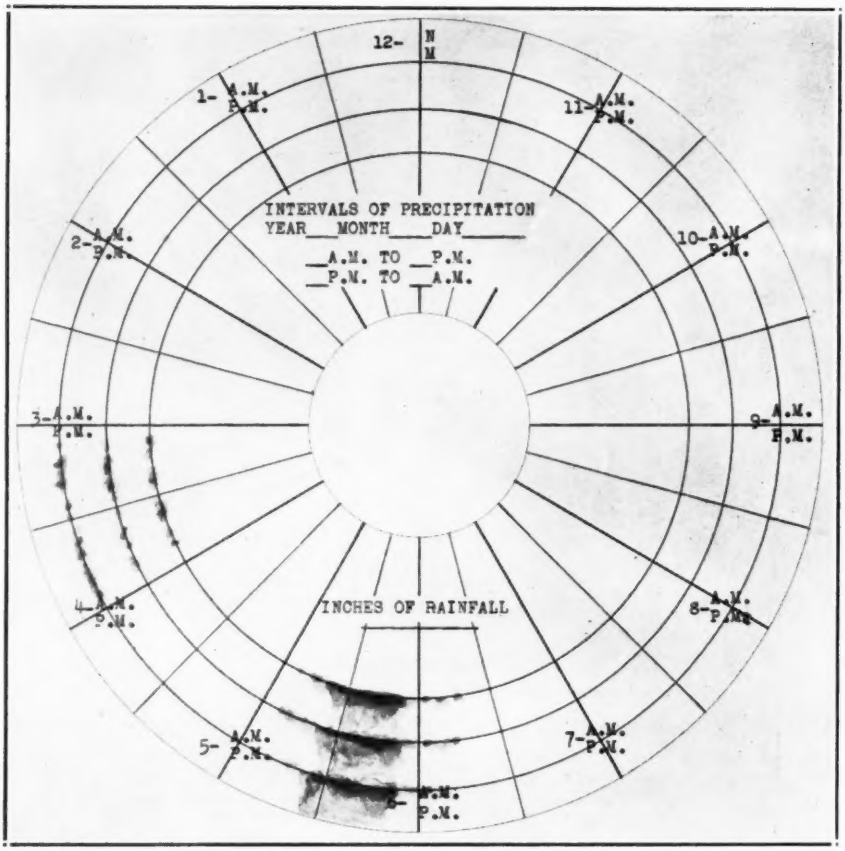

FIG. 2. A record sheet for the rain-recording instrument. Between 3 and 4 o'clock is an illustration of the record left by a light rainfall. The record of a heavier rainfall between 5 and 6 o'clock appears as a streaming of color from the arcs of the 3 concentric circles made with indelible lead.

pass beneath this opening in the cover. When it rained, the water drops fell through the hole in the cover and stained the indelible lines on the portion of the record sheet that at that time was exposed beneath it. The record of the duration of rainfall appeared as colored dots for light rains and as washed streaks of color for heavier rains (Fig. 2). The maximum time-error which might occur for any interval of rainfall was 15 minutes at the beginning and end of the period of precipitation. However in the particular investigation for which this instrument was devised, it was sufficient to know that rain did or did not occur within hourly intervals. By changing the record sheets once every 12 hours and indicating upon each sheet the time when it was placed in the instrument, a complete record of the time and

duration of any rainfall was secured. The amount of rainfall for a 12-hour period was obtained from the reading of a standard rain gauge and noted on the proper record sheet.

The recording device was placed in such a position that it tipped slightly forward; thus no water collected upon the paper sheets but ran off at once, leaving a clearer and sharper record than would otherwise be obtained. It is essential to have the cover of the box raised sufficiently above the disk so that water drops clinging to the edge of the slit will not extend to the revolving record sheet and moisten the indelible lines after the rain has ceased. It is obvious that the accuracy of the records will be increased directly in proportion as the opening in the cover through which the rain drops fall is made narrower.

This instrument has been used for 2 years and is still in good working condition.

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THE ISOLATION OF A TOXIC SUBSTANCE FROM THE CULTURE FILTRATE OF TRICHODERMA[1, 2]

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(Accepted for publication Jan. 17, 1936)

The parasitic action of the fungus *Trichoderma lignorum* (Tode) Harz on *Rhizoctonia solani* Kühn and other soil fungi has been described in previous papers,[3] and some of the properties of a lethal principle that is instrumental in this action have been studied.[4] This is a preliminary note on the isolation of a crystalline constituent of the lethal principle produced by a culture of Trichoderma.

In general, the methods of obtaining and testing the material toxic to Rhizoctonia were the same as those used before.[4] A nonpigmented culture was used, however, since the lethal effects of its culture filtrate lasted longer than those of the pigmented culture previously studied. The "shaking-culture" method described by Kluyver and Perquin[5] proved very useful in preparing large amounts of potent filtrate.

The toxic effect of this filtrate on Rhizoctonia can be removed by extraction with chloroform in a separatory funnel. The solvent is conveniently used in three portions, each equal to 0.1 of the volume of the filtrate. After distilling the chloroform from the extract, the residue is taken up in a small amount of hot benzene, or 95 per cent alcohol, from which, on cooling, silky white needles crystallize. This material is most satisfactorily recrystallized from benzene or alcohol.

The factors that determine the amount of crystalline material produced are not yet completely recognized. This problem is complicated by the decomposition of the material during and after growth. The highest yields (up to 70 mg. per liter of filtrate) have thus far been obtained when the fungus was grown for 2 days under the following conditions: strong aeration, high acidity of the medium (pH 3.0–4.0), ammonium tartrate as the nitrogen source.

[1] Paper No. 341, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

[2] This research was supported by a grant in aid from the National Research Council to H. S. Fawcett, under whose supervision it was carried out.

[3] Weindling, R. *Trichoderma lignorum* as a parasite of other soil fungi. Phytopath. **22**: 837–845. 1932.

[4] Weindling, R. Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. Phytopath. **24**: 1153–1179. 1934.

[5] Kluyver, A. J., and L. H. C. Perquin. Zur Methodik der Schimmelstoffwechseluntersuchung. Biochem. Ztschr. **266**: 68–81. 1933.

The substance in the crystalline form obtained in these studies is lethal to Rhizoctonia hyphae up to a dilution of 1 in 300,000 parts, which is about $\frac{2}{3}$ the toxicity of $HgCl_2$ under the conditions of these tests. A yellowish brown gum obtained upon evaporating the mother liquors to dryness has varying lethal effects, the maximum being the same as that of the crystals. In addition, it inhibits the growth of Rhizoctonia hyphae up to a dilution of one in three millons. The gum showed no tendency to crystallize, and has not been further investigated. The crystals, as well as the gum, are toxic to Trichoderma, but the minimum lethal dose is about 40 times that for Rhizoctonia. The dilution series for testing the substances has been made from alcoholic solutions, since the toxic substances are very sparingly soluble in water. Alcohol alone is not toxic at the dilutions used.

Composition and Properties of the Crystalline Substance. The substance is a compound of carbon, hydrogen, nitrogen, sulphur, and oxygen, possibly $C_{14}H_{16}N_2S_2O_4$. The determination of its molecular weight has offered some difficulties owing to the insolubility of the substance and its instability at higher temperatures. At 165° C. it begins to decompose, forming a yellow oil, and at about 185° C. it melts. The decomposition point of the substance has been determined according to a method devised by Bruce.[6] This method consists of determining the lowest temperature at which a given substance decomposes within 1 second after insertion of the melting-point tube into the bath. The results from this method agree ((within 1 or 2 degrees) with those obtained on the Dennis bar.[7] The decomposition point of the crystals after one crystallization from ethyl alcohol was 217°, and 221° C. after one crystallization from benzene. The same materials after 3 successive recrystallizations from alcohol or from benzene had decomposition points of 219° and 222° C., respectively. The substance is moderately soluble in acetone and chloroform, less soluble in hot benzene and in hot ethyl alcohol, and still less soluble in hot methyl alcohol. It is sparingly soluble in cold alcohol and ether. It is soluble in water, although to a very limited extent. It is strongly levorotatory, $[\alpha]_D^{19°} = -239°$ in chloroform.

The substance is easily altered. In alkaline solution it instantly decolorizes potassium permanganate with the formation of a green color. In spite of its nitrogen content, it has no basic properties. To 5 mg. dissolved in a few drops of warm acetone, was added $\frac{1}{2}$ cc. of 0.5 N hydrochloric acid; on cooling, the substance separated unchanged. After 15.2 mg. were boiled for one hour in 5 cc. of 3 N hydrochloric acid and extracted with chloroform, 13.5 mg. of a yellowish gum were recovered, but none of the original material could be crystallized from benzene or alcoholic solutions of this gum. 15.7

[6] Bruce, W. F., Organic synthesis. (In press.)

[7] Dennis, L. M., and R. S. Shelton. An apparatus for the determination of melting points. Jour. Amer. Chem. Soc. **52**: 3128–3132. 1930.

mg., dissolved in 15 cc. of ether, was shaken with 3 cc. of 1 per cent aqueous potassium hydroxide, then washed free of alkali. The ether-soluble residue weighed 15.0 mg., showing that almost nothing had been taken up by the alkali. However, afterwards it was possible to isolate only 5 mg. of crystals, indicating that this mild treatment had caused some alteration. Boiling with 5 per cent aqueous potassium hydroxide rapidly splits out sulphur, as shown by the formation of a black precipitate of lead sulphide upon the addition of a few drops of lead acetate solution.

Composition Found by Analysis. C 47.75 per cent, 48.07 per cent;[8] H 4.36 per cent, 4.43 per cent;[8] N 8.25 per cent, 8.27 per cent;[8] S 19.4 per cent.[9]

Composition Calculated for $C_{14}H_{16}N_2S_2O_4$. C 49.38 per cent; H 4.73 per cent; N 8.23 per cent; S 18.82 per cent.

Optical Rotation. $[\alpha]_D^{19°} = -\frac{2.23 \times 1000 \times 3}{28} = -239°$.

Molecular Weight Determination. Diphenyl amine was the only substance available in which the toxic crystals were reasonably soluble, and the melting point of which was low enough to insure no decomposition. Even diphenyl amine, at its melting point, did not dissolve more than about 6 per cent of its weight of the crystals.

In 24.8 diphenyl amine, 0.90 mg. substance lowered the melting point 0.9° C.[10]

Molecular weight. $\frac{100 \times 0.9 \times 86}{24.8 \times 90} = 347.$

Calculated for $C_{14}H_{16}N_2S_2O_4 = 340.$

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[8] By A. Schoeller, Berlin.

[9] By Kurt Eder, University of Illinois.

[10] Value of 86 for molecular freezing point lowering of diphenylamine from: Chemical Rubber Company, Cleveland. Handbook of Chemistry and Physics. 20th ed. Chemical Rubber Publishing Co., Cleveland, O. 1935.

APPLE POWDERY MILDEW

C. E. Berwith

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Among the distinctive characteristics of the powdery mildew of the apple, caused by *Podosphaera leucotricha* (E. & E.) Salm., is the far greater severity of the disease in districts on or near the Pacific coast of North America, than at corresponding latitudes in the central and eastern parts of that continent. It has been of interest, therefore, to study some of the conditions affecting the germination of conidia and the inception of infection and to examine under local conditions in California the mode of perennation heretofore studied in detail only by Woodward[1] in England.

GERMINATION OF CONIDIA

Relation of Temperature. Woodward states that the conidia germinate best between 10° and 15° C. (up to 3 per cent in water), while 20° to 22° C. is most favorable for growth of the germ tubes.

The percentage germination in the writer's experiments has been low at best, as was the case with earlier workers. Dry storage of conidia for periods of 1, 2, and 14 days at temperatures from 1 to 10° C., did not seem to affect viability as indicated by subsequent germination (1 to 6 per cent) at 20° C. When freshly collected conidia were shaken immediately onto dry glass slides in moist chambers at different temperatures, a fairly definite optimum was indicated at 19° to 22° C. (Table 1). Each figure in the table represents a count of 100 spores for each of 10 trials or 1000 spores for each temperature. At other temperatures below and above those shown in the table there was no germination.

TABLE 1.—*Percentage germination of conidia at different temperatures*

Temperatures °C.		7	10	13	16	19	22	25	28	31
Time interval	6 hrs.	0	1	1	2	3	3	2	0	0
	48 hrs.	0	1	1	2	4	5	2	0	0

Relation of Humidity. Conidia placed on dry glass slides in dry containers at temperatures from 1° to 34° C. failed to germinate in any case. The conidia at 1° C. were viable after 2 weeks, while those at 31° and 34° were shrivelled after 6 hours. The spores held at 13° and above for 24 hours did not germinate when exposed to a favorable humidity and temperature.

[1] Woodward, R. C. Studies on *Podosphaera leucotricha* (Ell. & Ev.) Salm. I. The mode of perennation. Brit. Mycol. Soc. Trans. **12**: 173–204. 1927.

In another experiment, conidia on dry slides were placed in chambers over such concentrations of sulphuric acid as to maintain relative humidities of approximately 1.4, 30, 70, 90, and 100 per cent. Only the last of these permitted germination. Barium chloride and other chemicals in solution were used in a similar way with similar results, no germination occurring at humidities of 90 per cent or below. It is apparent then that a high atmospheric humidity is essential for the germination of conidia. However, when conidia were mounted in hanging drops of water, the germination, if any, was very low, never over 1 per cent. In raised drops of water no germination was obtained. Likewise, when conidia were mounted in hanging drops of water containing extracts of apple leaves or fruits, orange juice, sucrose (1 and 5 per cent), or Czapek's solution (1 per cent) no germination resulted, while controls in distilled water gave 1 per cent germination.

INFECTION OF APPLE LEAVES

Young seedling apple leaves were detached, inoculated with conidia, and placed with the petioles in vials of water in chambers at different temperatures and humidities. The humidities were maintained as indicated above by the use of different concentrations of sulphuric acid.

No infection was obtained with any humidity at 1° to 10° C. or at 28° C., or at favorable temperatures when the humidity was 90 per cent or lower. At 13°, 16°, 19°, 22°, and 25° C., and at approximately 100 per cent humidity, infection was evident in 45 to 48 hours and conidiophores were produced in 5 days.

Relation of Age of Leaf and Wounding to Infection. To study the relation of age of leaf to susceptibility, leaves were marked soon after emergence from the terminal bud and inoculated at different intervals thereafter. With this method, and using rather slow-growing seedling plants, fair to good infection was obtained on leaves inoculated 1 day after marking, but none on leaves inoculated after 5 days. By abrading the leaf surface with powdered carborundum[2] prior to inoculation, infection was obtained on the abraded portion of leaves (8 of 10 plants) inoculated 9 days after marking, but not elsewhere on the same leaves. It is apparent that the resistance of leaves increases rather rapidly with increasing age and that this resistance can be partially broken down by slight abrasion of the leaf surface.

PERENNATION

Many investigators of this disease have held the view that the perithecia play little or no part in the annual cycle of the fungus and that it survives the winter in or on the buds. It is only recently, however (Woodward),

[2] Rawlins, T. E., and C. M. Tompkins. The use of carborundum as an abrasive in plant virus inoculations. (Abstract) Phytopath. **24**: 1147. 1934.

that a comprehensive study of this problem has been made. The evidence against the participation in the cycle by the perithecia, though of a negative sort, is nevertheless impressive and seems now to be conclusive. Several attempts in the course of this work to germinate the ascospores, both in the presence and absence of apple leaves, were unsuccessful, as were the similar efforts of earlier workers. On the other hand, the mycelium, haustoria, and sometimes conidia are readily demonstrated to be rather deep-seated in the resting terminal and lateral buds of shoots that have become completely invested by the fungus. Both free-hand and microtome sections were made from buds (mostly Yellow Newtown) collected in several different years in July, September, November, December, and February. The fungus is often seen midway or below in the interstices of the buds of the sort that regularly open in spring, with all parts completely covered with mildew. The appearance of the mycelium in the buds during the winter months suggests that the fungus probably survives in the mycelial form.

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THE RELATION OF THE AGE OF NEEDLES OF PINUS STROBUS TO INFECTION BY CRONARTIUM RIBICOLA

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The question of the susceptibility of white pine needles of different ages to *Cronartium ribicola* Fisch. is of interest not only academically and scientifically, but also in epiphytotological and phenological studies. In the West it appears to have been established beyond question that in *Pinus monticola* not only are the needles more susceptible to infection in their second season (when they are a little over a year old), but also, that a plurality, if not a majority, of the cankers occur on wood that was a year old at the time of infection (2). In the East, on the other hand, where *Pinus strobus* is the tree concerned, there is some question in this matter, with the result that it has been impossible with certainty to give definite dates in the epiphytotological history of the disease or to correlate waves of infection with meterological data in phenological studies.

Many, if not most, of the workers in the East believe that the same condition holds for *Pinus strobus* as for *P. monticola*—that one-year-old needles are more susceptible to the fungus than are new needles in their first year of activity. This may be true, but in the writer's opinion, the question has not yet reached the incontrovertible stage. On the other hand, the writer is not ready to say that the results presented herewith give the final answer. He does feel, however, that they cannot lightly be dismissed and that they must be satisfactorily explained before the opposing view becomes established. It is hoped that, at the least, these results will provoke caution against becoming dogmatic about the matter, until enough evidence one way or the other is presented satisfactorily to answer the question.

RESULTS OF INOCULATIONS OF POTTED SEEDLINGS, NORTH CONWAY, NEW HAMPSHIRE

In the early days of white pine blister rust investigations by the Office of Forest Pathology, the writer assisted with the inoculation of hundreds of potted white pine seedlings in New Hampshire. These seedlings, ranging in age from one to 10 years and mostly under 5 years, were collected in the woods and transferred with very little disturbance of the root system to paper cups. Various methods of inoculating with sporidia were used, and when a satisfactory technique had been developed, the success of inoculation was consistently between 50 and 100 per cent.

The conclusions regarding these experiments were reported as follows. In the inoculations of 1918 and 1921 seedlings, if anything, there were more

individual infections on current season's needles than on fully grown leaves (5, p. 149), and again, "77 per cent of the first-year seedlings became infected, whereas only 58 per cent of the older ones (most of which were over 3 years old) became infected" (6, p. 502). Colored plate I of this latter article shows about equal susceptibility of the two ages of needles. Of the 1922 inoculations, seedlings of all years except the first were successfully inoculated, thus contradicting the 1921 results (6, p. 505).

Clinton and McCormick (1) found that a larger percentage of 1-year-old seedlings than older ones became infected as the result of their inoculations.

RESULTS OF THE INOCULATION OF OLDER NATURAL PINES, NORTH CONWAY, NEW HAMPSHIRE

In 1922, at North Conway, N. H., pines from 5 to over 30 years of age, growing undisturbed in their natural habitat, were inoculated. These results have been reported (4). As to the susceptibility of the needles, it was stated—"None of the inoculations of 1920 needles, 18 per cent of those of 1921 needles and 19 per cent of those on the 1922 needles developed leaf spots. None of the inoculations on 1920 needles, 9 per cent of those on 1921 needles, and 8 per cent of those on 1922 needles resulted in twig cankers" (6, p. 507). The pertinent conclusions from the examination of 127 other cankers were as follows (*loc. cit.*) :—"Ninety-five per cent of these cankers were on 1922 wood and 5 per cent on 1921 wood. These results confirm the above statement that both 1- and 2-year-old needles are susceptible. They do not, however, give any informaton on the relative susceptibility of 1921 and 1922 needles, for no record was kept of the relative percentages of the 1921 and 1922 originally inoculated. The most that can be said is that no indication was encountered that 2-year-old needles of *Pinus strobus* are more liable to infection than 1-year-old needles."

RESULTS OF INOCULATIONS OF PLANTED PINES AT NEWCOMB, NEW YORK

Beginning in 1929, for 4 seasons the writer made inoculations of pines set out at Newcomb, N. Y., for the purpose of testing the resistance of the offspring of an apparently immune parent tree. The trees were 6 years old when the first inoculations were made and 9 years when the last ones were done. In these tests infected black currant leaves were wrapped around the needle-bearing portion of the pine twigs and fastened in place. The entire needle-bearing portion of a branch was so treated. Thus, in every unit inoculated, there were needles of current, second, and third seasons of activity, and in some cases, some of 4 seasons.

In all, 389 test and control trees were inoculated and about 56 per cent became infected. The data on the relative susceptibilities of needles of different ages are given below in terms of infection of the internodes and number of cankers an internode.

TABLE 1.—*Data on inoculations of planted pines at Newcomb, New York*

Age of needles inoculated	Internodes			Cankers	
	Total no. inoculated	No. infected	Per cent infected	Total no. cankers	Ratio of cankers to no. internodes inoculated
Current season	3034	416	13.7	497	1 to 6
Second season	1392	163	11.7	184	1 to 8
Third season	448	36	8.0	40	1 to 12
Fourth season	58	3	5.0	3	1 to 20

In these experiments, the year-old needles were somewhat less susceptible than those of the season current with the inoculation, and the ratio of cankers to internodes inoculated was lower, the older the needles at the time of inoculation.

OBSERVATIONS OF NATURAL INFECTIONS ON THE HEWITT AREAS IN NEW YORK

The so-called "Hewitt Areas" in New York are those parcels of land acquired by the State under the Hewitt Act of 1931 for afforestation purposes. In the course of studies concerning the blister-rust control program on these areas over the southern tier of counties from the Catskills to Lake Erie in 1934, observations were made on the cankers found, which were necessarily few in number because of the eradication of Ribes.

The trees on most of the areas studied were planted in 1930 or 1931. These studies were made in the summer of 1934, before 1934 infections could be seen. Hence, the cankers studied could have resulted from infection in one of only 4 years at the most, and in one of 2 years, in some cases. One or 2 areas planted in 1932 were searched—areas in which the only opportunity for visible cankers to have had their inception could have been in 1932—but on these areas, unfortunately, there were only very few cankers to be found, perhaps a half dozen to several thousand trees examined. The criteria used for determining the year of origin of the cankers were as follows.

(A) If on any year's wood there was only discoloration of the bark, it was considered to be due to the 1934 growth of the fungus, and, therefore, the canker originated in the summer of 1933. Such discolorations on 1933 wood were from $\frac{1}{8}$ to $\frac{3}{4}$ inch long. Here it may be realized that it is possible that discolorations on 1932 wood were of 1932 instead of 1933 origin, as they are here considered, because the infection spots on the needles may have been so far from the twig that it took a year for the fungus to reach the stem.

(B) If on any year's wood there were only two zones (a) discoloration (1934 growth of the fungus) and (b) a pycnial zone (the 1933 growth), the

canker was considered to be of 1932 origin. These cankers measured from $\frac{1}{8}$ to $2\frac{7}{8}$ inches, with one 3 inches and another $3\frac{1}{8}$ inches long.

(C) If on any year's wood, there were three zones (a) discoloration (the 1934 growth of the fungus) (b) a pycnial zone (the 1933 growth) and (c) old pycnial scars and/or aecial scars of 1934 (1932 growth of the mycelium), then the canker was considered to have originated in 1931. These cankers were found to measure from 3–9 inches in length.

On the basis of the foregoing criteria, the cankers found were distributed as shown in the following table.

TABLE 2.—*Distribution by years of cankers on Hewitt areas in New York*

Year of wood on which canker was found	Stage of development of canker		
	Discoloration only ($\frac{1}{8}$″ to $\frac{3}{4}$″)	Discoloration and pycnial zones (mostly $\frac{7}{8}$″ to $2\frac{7}{8}$″)	Discoloration, pycnial and aecial zones (3″ to 9″)
1933	10	—	—
1932	7	71 (current 1932 infections)	—
1931	2	51 (1932 infections of 2nd season needles)	330 (current 1931 infections)
1930	—	14 (1932 infections of 3rd season needles)	145 (1931 infections of 2nd season needles)
1929	—	—	41 (1931 infections of 3rd season needles)

The foregoing data may be presented in another form, as follows.

Year in which infection took place (origin of canker)	Season's needles infected		
	Current	Second	Third
1933	10	7	2
1932	71	51	14
1931	330	145	41
Totals	411	213	57
Ratio	7.21 to	3.72 to	1

EXPOSURE OF POTTED PINES TO NATURAL INFECTION AT TUPPER LAKE, NEW YORK

In 1931, 17 pines, potted that spring, were exposed during August underneath some cultivated black currant bushes at Tupper Lake, New York.

They were then transplanted for observation, to gardens in Warrensburg, New York, and Providence, Rhode Island, where there was little likelihood of occurrence of further infection.

TABLE 3.—*Results of exposure of potted pines to natural infection at Tupper Lake, New York*

Location of pines after transplanting, and number	Current season's needles		Second season's needles	
	Internodes infected	No. cankers	Internodes infected	No. cankers
Warrensburg (12)	33	54	38	65
Providence (5)	10	10	0	0
Totals	43	64	38	65

The year-old needles of the 12 pines left at Warrensburg were somewhat more susceptible than the first-season needles. Why there should be the difference between the 2 lots of pines is difficult to understand, for they were all exposed at the same place for the same period, and the 5 removed to Providence were selected at random. The pines taken to Providence apparently were not placed in such a favorable situation, for they did not grow well and died within 2 or 3 years. Why cankers should have developed on the internodes of the current season of inoculation and none on the year-old internodes of the pines at Providence is likewise a mystery, unless the pines were in such a poor location that the needles became weakly and, therefore, inhibited the mycelium of the fungus from reading the stems.

DISCUSSION

From the foregoing results of observations and experiments of different sorts over more than 15 years in different localities, it can be seen that in most cases the young needles of the first season turned out to be at least somewhat more susceptible to infection by the blister-rust fungus than the year-old needles of the second season of growth. The writer is not, however, insistent upon maintaining the implied thesis. The numbers of plants were, in most cases, small. The observations were, in some cases, limited in scope. The technique may, in some cases, have been faulty or unreliable. There is always a suspicion of artificial inoculations and a question as to the value of the results of these in explaining natural phenomena. The least that can be said, however, is that one should go slowly in advocating the opposite thesis with regard to *Pinus strobus* until valid criticism can show that these results are unreliable or until results to the contrary are uniformly obtained from inoculations and observations under natural conditions.

In connection with inoculation experiments with potted pines, one possible criticism should be avoided in the planning of the work. By this is meant the possibility of objection to the use of pines potted in the same season in which they are to be inoculated or exposed. It is readily observed that transplanted pines do not make such vigorous growth in the season in which they are transplanted, if they make any considerable growth at all. New growth in such pines may be 3 to 5 inches long, whereas the growth on the same trees of the season before, or the season following, would measure 6 to 9 inches in length. The needles on the new wood in the season of transplanting are invariably shorter than before or after. It seems to have been demonstrated that the blister-rust fungus is more likely to produce the disease in vigorous than in weakly growth (3, p. 483). Therefore, until evidence to the contrary is produced, it may always reasonably be objected that the current growth of pines transplanted in the same season in which the experiments are carried on, may possibly have its susceptibility to *Cronartium ribicola* reduced by its lack of normal vigor.

It just so happens that the only set of experiments set forth in this paper, in which the second-season needles were more susceptible than the first-season needles (in the potted pines exposed at Tupper Lake and transplanted at Warrensburgh, New York), was concerned with pines potted in the spring of the same season in which they were exposed to infection.

SUMMARY

It is still an open question whether the first-season needles of *Pinus strobus* are more or less susceptible to infection by *Cronartium ribicola* than the second-season needles (one-year-old).

The results (1) of the artificial inoculation of potted white pines, of older pines in their natural environment and of young planted pines, (2) of the exposure of potted pines to natural infection, and (3) of observations of cankers on young planted pines, are to the effect that the current season's needles are to a greater or less degree more susceptible to *C. ribicola* than the second season's needles.

It is suggested that, in experiments planned for the purpose of settling this question the use of pines potted in the same season in which they are to be inoculated or exposed to natural infection, may justly be open to criticism.

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PHYTOPATHOLOGICAL NOTES

Sclerotium rolfsii as a Disease of Nursery Apple Trees.—In August, 1935, some yearling apple trees with diseased roots were received from a Maryland nursery for diagnosis of the trouble. By means of the usual culture methods a pure culture of *Sclerotium rolfsii* was readily obtained. Diseased apple trees from other nurseries also yielded the same fungus. From apparently diseased root tissue taken later in the summer, however, cultures were obtainable from only a very small percentage of the material, if at all. In one case, material from which the Sclerotium fungus had been previously isolated and that still showed macroscopic characters of the disease, gave negative results for cultures, and, when placed in moist chambers, developed sclerotia in only 1 of 30 roots. Repeated efforts to isolate the fungus from trees whose roots had been dead and left undisturbed in the soil for a month or more, gave negative results. This seems to indicate that, frequently, the causal fungus dies soon after killing the host. The early dying of the fungus in the tissues it has killed suggests the possibility that the disease may be of more frequent occurrence than has hitherto been suspected, and that failure of the writer and other workers to isolate the fungus more frequently may be due to its early disappearance.

The following experiment was conducted to test the correctness of the field observation on the pathogenicity of this fungus. On February 15, 1936, roots from apple nursery stock were inoculated with a pure culture of *Sclerotium rolfsii* by placing about them sclerotia growing on un-hulled oats that had previously been inoculated. The apple roots were then packed in moist sphagnum and held at room temperature. At the expiration of a month, 18 of the 20 roots were infected with *S. rolfsii* and extensive killing was in evidence about the infections. From these diseased roots, a fungus similar to that used as inoculum was reisolated. This test confirms the field observation that *S. rolfsii* readily and rapidly kills the roots of young apple trees when conditions are favorable for its growth.

The disease on apple nursery stock usually may be recognized in the early stages by a web of white mycelium on the tree at the soil level and on the soil around the tree. Within a few days the white mycelium disappears and masses of brown sclerotia (Fig. 1) about the size and color of mustard seeds may be seen on the collar of the tree and on the soil immediately adjacent to the tree. By the time sclerotia are formed the bark of the tree is killed about the collar, effecting complete girdling, which soon results in death of the top. The fungus is confined largely to root tissue, though it may advance up the stem and kill it slightly above the surface of the soil. The bark, especially of roots, is the main tissue invaded and is very quickly killed.

FIG. 1. Budded apple tree girdled in the nursery row by *Sclerotium rolfsii* during the first summer. Arrows point to sclerotia (mature at right, immature at left).

Observations thus far indicate that the disease is more frequent on relatively moist than on dry soil. Trees killed by this fungus do not occur in large groups but are scattered here and there over the affected area. This is probably due to limited distribution of sclerotia at the proper place on the

plant for infection. Considering the fact that the disease has come to the writer's attention in several widely separated apple nurseries during the summer of 1935, it should be recorded as a nursery disease.

Under certain conditions, there may be an appreciable loss of young apple trees. In one nursery, losses have occurred for the past 8 years from a disease apparently the same as that now known to be associated with the Sclerotinum fungus disease—the losses amounting to as much as 5 per cent of the trees.

Turner[1] has reported the occurrence of *Sclerotium rolfsii* on young apple trees in the orchard.—J. S. COOLEY, Bureau of Plant Industry, U. S. Department of Agriculture.

Invasion of Cotton Seed by Bacterium malvacearum.[1]—The percentage of cotton-seed contamination due to invasion by *Bacterium malvacearum* E. F. S. is usually low in dry weather, but, under favorable conditions, the seedling infection resulting from internal contamination may reach 20 per cent or more. The bacteria gain entrance to the mature seed through the basal end at the chalaza.

The ovule is surrounded by two seed coats observed by Barrit,[2] Brown,[3] Reeves and Valle.[4]

If a delinted cotton seed that has been soaked in water is placed under a low power microscope, a circular opening through a portion of the outer seed coat at the chalazal end can be seen (Fig. 1, A). The part of the seed coat exposed by this opening is a cap-like structure composed of pigment-bearing cells and large intercellular spaces. This structure is a portion of the layer designated by Brown (2) as pigment-bearing tissue.

While the seed is immature, the smallest intercellular spaces of the cap are .9 μ wide, but by the time the seed has ripened and dried, the spaces have increased to 7.6 to 19 μ wide.

The following tests were designed to determine the porosity of the intercellular spaces of the basal cap:

Test 1. Untreated cotton seeds were soaked in eosin water. Examination of the seed showed that the path of entrance of the eosin-strained water was through the basal cap.

[1] Turner, T. W. Pathogenicity of *Sclerotium rolfsii* for young apple trees. (Abstract) Phytopath. **26**: 11. 1936.

[1] Approved for publication by the Oklahoma Agricultural and Mechanical College Experiment Station.

[2] Barritt, N. W. Structure of the seed coat in Gossypium. Ann. Bot. **43**: 483–489. 1929.

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[4] Reeves, R. G. and C. C. Valle. Anatomy and microchemistry of the cotton seed. Bot. Gaz. **93**: 259–277. 1932.

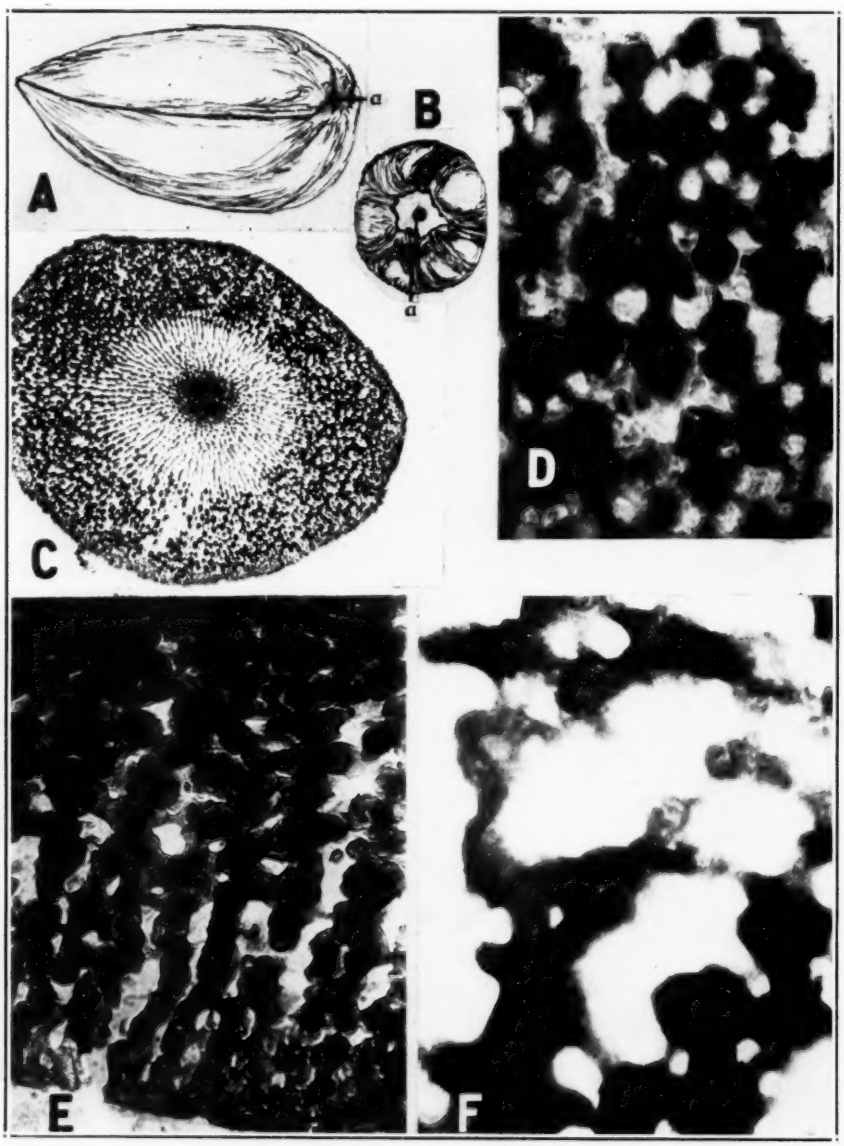

FIG. 1. A. Side view of delinted and water-soaked cotton seed, showing rupture in seed coat at chalazal end and exposed basal cap (*a*). ×4¾. B. End view of delinted cotton seed, showing the basal cap (*a*) at the chalazal end under extreme conditions of pulling apart of the outer seed coat. ×4¾. C. Cross section of cap. ×100. D. Enlarged portion of C. ×430. E. Longitudinal section through cap structure. ×430. F. Enlarged portion of E. ×900.

Test 2. Delinted cotton seed, with the chalazal end uppermost, were treated with water-soluble starch for 16 hours. Sections of the tissue from the cap were treated with potassium iodide. The starch grains were seen lodged underneath the cap and in the intercellular spaces.

Test 3. This test was conducted in the same manner as test 2. *Bacillus subtilis* in a water blank was used instead of starch. Sections from these seeds showed the bacteria in the intercellular spaces of the cap and along the perisperm layers of the inner seed coat.

Test 4. *Bacterium malvacearum* was used to inoculate the seed. Sections from these seeds showed bacteria underneath the basal cap and in the intercellular spaces of the cap.

Having determined that bacteria could enter the seed through the basal cap in the presence of water, it is necessary to know how and where the conditions exist for seed contamination to take place. This is explained by the fact that rain washes innumerable bacteria to the ground from the foliage of infected leaves. Millions of the organisms are suspended in the run-off water between the rows of cotton.

Evidence shows that the organism remains active in the run-off water for a period of 44 to 56 hours.[5] Tests show that the seed of open bolls soaked in bacteria-laden water carry both internal and external contamination.

When once the bacteria enter the mature seed, they apparently become inactive and remain so until the germination processes set in. The basal cap adheres to the cotyledons and is carried up as the plant emerges from the soil. The soil water may carry the bacteria to the seed leaves and hypocotyl, or rain and dew may wash the organism onto the leaves and stems, thus inducing primary seedling infection.—GERTRUDE TENNYSON, Oklahoma Agricultural and Mechanical College, Stillwater, Okla.

Fusarium lateritium v. fructigenum in Relation to Wilt of China Aster.—A recent article by Riker and Jones[1] described studies at the University of Wisconsin on the pathogenicity to China aster, *Callistephus chinensis* Nees, of various Fusarium strains from this country and abroad.

One of the strains from Germany was called *Fusarium lateritium* Nees v. *fructigenum* (Fr.) Wr. This strain was furnished and identified by H. W. Wollenweber who numbered it 4221. The first transfer was received in Wisconsin in the fall of 1930. Subsequent trials, always started by direct transfer from the culture obtained from Germany, proved not only that it was pathogenic to China aster but that the aster strains, susceptible and

[5] Rolfs, F. M. Bacterial leaf spot of cotton. Oklahoma Agr. Expt. Sta. Rept. 1930–32: 280–283.

[1] Riker, R. S., and L. R. Jones. Fusarium strains in relation to wilt of China aster. Phytopath. 25: 733–747. 1935.

resistant with respect to the type strain of *F. conglutinans* Wr. v. *callistephi* Beach, were respectively susceptible and resistant with respect to 4221. A fresh transfer of 4221 was requested and received from Germany in the spring of 1932. It behaved similarly to the first transfer.

Recent examinations by W. C. Snyder and H. W. Wollenweber of 4221, as carried in culture both in this country and in Germany, have suggested that the original 4221 was a mixture of *Fusarium lateritium* v. *fructigenum* and *F. conglutinans* v. *callistephi*.

This new evidence makes it probable that the results obtained in the use of 4221 were due to *Fusarium conglutinans* v. *callistephi*. Consequently, the paper by Riker and Jones[1] should be so interpreted. The Fusarium strains shown to induce a wilt of aster essentially similar to that caused by the type strain of *F. conglutinans* v. *callistephi* are, therefore, all classified, according to standard methods, in the section Elegans.—REGINA S. RIKER, University of Wisconsin, Madison, Wis.

Viability and Longevity of Chlamydospores of Ustilago crameri.—Chlamydospores of *Ustilago crameri* Kcke. will germinate even before they are morphologically mature, and some have retained their viability for at least 62 years. These facts were obtained in investigating the development and cytology of the organism. To determine the ability of very young spores to germinate, they were taken from sori of various ages, beginning with the emergence of the millet heat until the host plant matured. Germination tests were made both in water and 2 per cent sugar solution. A considerable number of spores germinated before they were morphologically mature, when there was still a considerable amount of mycelium in the gall and spore mother cells and spores were present in about equal quantities. Obviously, no rest period is necessary, and the spores appear to be physiologically mature even before they are morphologically so.

It has been known that chlamydospores of *Ustilago crameri* would retain their viability for several years. Hoffman and von Liebenberg, according to de Bary,[1] stated that they retained their viability for $5\frac{1}{2}$ years, and Vasey[2] found them viable after 3 years.

The writer tested the longevity of spores stored at 7° to 9° C., 16° to 20° C., and 20° to 25° C. A fairly high percentage retained their viability for three years. Tests also were made of spores taken from Rabenhorst, Fungi Europaei, Cent. 19, 1874. In several tests about 1 per cent of these spores germinated, indicating that they had retained their viability for at least 62 years. It is probable that the spores actually were 64 years old, as they

[1] Bary, A. de. Comparative morphology and biology of the fungi, mycetozoa, and bacteria. 625 pp. Oxford, 1887.

[2] Vasey, H. E. Millet smuts and their control. Colo. Agr. Exp. Sta. Bul. 242. 1918.

apparently had been collected by R. Körnicke at Poppelsdorf, Germany, in 1872.—C. S. WANG, University of Minnesota, St. Paul, Minn.

A Race of Crown Rust to Which the Victoria Oat Variety is Susceptible.[1]—The oat variety Victoria (C. I.[2] No. 2401) recently was reported by the senior author[3] as being resistant to 37 physiologic races of crown rust (*Puccinia coronata avenae* Eriks. and Henn.). Since this report was written, the junior writer has found Victoria susceptible to a collection of crown rust made on December 28, 1935, at San Antonio, Texas, by Wallace T. Butler, Agent, Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, U. S. D. A. Using inoculum obtained from specimens of these infected Victoria plants, the senior writer identified this collection as a race of crown rust new to North America and heretofore not reported from any other place. This new race is hereby designated as key number 41.

The reactions of 11 differential oat varieties (selected in 1931 by the senior writer and by B. Peturson, Dominion Rust Research Laboratory, Winnipeg, Manitoba, Canada, as standard differential varieties for identification of parasitic races of *Puccinia coronata avenae* occurring in North America) to races 1 to 33, inclusive, have been published by Murphy.[4] The reactions of these same varieties to races 34, 35, 36, and 37 were determined by the senior writer and published with his permission by Stakman, Levine, Christensen, and Isenbeck.[5] Peturson[6] described the reactions of the standard differential varieties to two new races A and B (later designated as standard races 38 and 39, respectively). W. L. Waterhouse, University of Sydney, Australia, has recently identified a new race, designated as number 40, for which the reaction of the differential varieties has not yet been published.

The reactions at approximately 70° F. of the standard differential varieties to race 41 are presented in table 1. The reactions of Victoria and Bond

[1] Journal Paper No. J-355 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project No. 72; and Paper No. 1439 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station, St. Paul, Minn.

[2] C. I. refers to accession number of the U. S. Dept. Agr., Bur. Plant Industry, Division of Cereal Crops and Diseases (formerly Office of Cereal Investigations).

[3] Murphy, H. C. Reaction of the Victoria oat variety to crown rust. Phytopath. **26**: 396–397. 1936.

[4] Murphy, H. C. Physiologic specialization in *Puccinia coronata avenae*. U. S. Dept. Agr. Tech. Bull. 433. 1935.

[5] Stakman, E., M. N. Levine, J. J. Christensen, and K. Isenbeck. Die Bestimmung physiologischer Rassen pflanzenpathogener Pilze. Nova Acta Leopoldina **3**: 281–336. 1935.

[6] Peturson, B. Physiologic specialization in *Puccinia coronata avenae*. Sci. Agr. **15**: 806–810. 1935.

TABLE 1.—*Host reaction to parasitic race 41 of Puccinia coronata on differential varieties of Avena spp.*

Differential varieties tested		Average infection type produced by race 41
Name	C. I. No.	
Ruakura	2025	1
Green Russian	2890	2
Hawkeye	2464	4
Anthony	2143	4
Sunrise	982	4
Green Mountain	1892	4
White Tartar	551	4
Red Rustproof	1815	3
Sterisel	2891	3
Belar	2760	4
Glabrota	2630	0
Victoria	2401	3
Bond	2733	0

(C. I. No. 2733) also are included in this table because of their outstanding adult resistance to epiphytoties of crown rust when growing under field conditions, and their seedling resistance to a large number of important races. Victoria is known to be resistant in the seedling stage to races 1 to 37, inclusive, and susceptible to race 41. Bond is known to be susceptible only to races 33 and 34. It is probable that additional crown-rust races will be isolated and identified solely on the basis of the differential reaction of either or both Victoria and Bond. For these reasons it seems desirable to include these two differential varieties among the standard varieties used in identifying all collections of crown rust, even though the 41 races discovered to date may be identified by using only the first 11 varieties listed in table 1.

The rare race 41 may not increase in prevalence and distribution and may even disappear, although with an increase in acreage of oat selections, such as described by Stanton *et al.*,[7] which are endowed with Victoria's resistance to other prevalent races, this new race could increase rapidly. Fortunately, Bond is completely resistant to race 41, thereby making available an immediate source of resistance to it. Hybrid selections combining Bond's resistance to crown rust and to smut with the resistance of certain high-yielding varieties to stem rust have been developed by Murphy, Stanton, and Coffman.[8] At present, then, for every known parasitic race of

[7] Stanton, T. R., H. C. Murphy, F. A. Coffman, and H. B. Humphrey. Development of oats resistant to smuts and rusts. Phytopath. **24**: 165–167. 1934.

[8] Murphy, H. C., T. R. Stanton, and F. A. Coffman. Hybrid selections of oats resistant to smuts and rusts. Jour. Amer. Soc. Agron. **28**: 370–373. 1936.

rusts or smuts affecting oats, an oat variety completely resistant to such race is known. So long as this situation exists, breeding for disease resistance in oats has a promising outlook.—H. C. MURPHY and M. N. LEVINE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, in cooperation with the Iowa and Minnesota Agricultural Experiment Stations, respectively.

THE TWENTY-EIGHTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

ATLANTIC CITY, NEW JERSEY

MONDAY, DEC. 28 TO THURSDAY, DEC. 31, 1936

The Society, as an affiliated organization, joins in the annual meeting of the American Association for the Advancement of Science which is to be held in Atlantic City from December 28, 1936, to January 2, 1937. A.A.A.S. General Headquarters will be at Haddon Hall, Registration Headquarters at the City Auditorium.

The AMBASSADOR HOTEL has been assigned as
SOCIETY HEADQUARTERS
The SESSIONS of the SOCIETY will be held in the
ATLANTIC CITY AUDITORIUM